Module 14: Blood Collection and Handling – DBS

Purpose

To provide the participants with skills to collect and handle dried blood spots (DBS) for EQA purposes

Pre-requisite Modules

- Module 6: Safety at HIV Rapid Testing Site
- Module 8: Blood Collection – Fingerprick
- Module 13: EQA

Module Time

1 ½ hours

Learning Objectives

At the end of this module, participants will be able to:

- Collect dried blood spots (DBS)
- Package and store DBS in a way to maintain specimen integrity
- Maintain DBS records
- Distinguish between valid and invalid DBS

Module Overview

Step	Time	Activity/ Method	Content	Resources Needed
1	5 min	Presentation	Module introduction	Slides 1-7
2	20 min	Presentation; Activity; Demonstration	Procedures for collecting, drying, packaging, and storing DBS	Slide 8-20; DBS supplies (for display and demonstration)
3	10 min	Presentation; Discussion	Valid vs. invalid DBS	Slides 21-29
4	50 min	Demonstration; Practice Exercise	Collecting DBS	Slide 30-32; DBS supplies (for practice)
5	5 min	Q&A	Summary	Slide 33

Material/Equipment Checklists

- PowerPoint slides or transparencies
- Overhead projector or computer w/LCD projector
- Prepared Flipchart – content outline
- One-pager job aid for finger prick
- Handout: Example Specimen Transfer Log for Re-Testing
- Materials and supplies required for collecting and handling DBS. Have enough sets for participant inspection, demonstration and hands-on practices.

Teaching Guide

Slide Number	Teaching Points
 *Customization Notes*	SIMPLIFY this module if participants already know how to collect and handle DBS. But make sure they all follow the standard procedures outlined in this module.
1	**Module 14: Blood Collecting and Handling (DBS)** DISPLAY this slide before you begin the module. Make sure participants are aware of the transition into a new module.
2	**What Is a Dried Blood Spot (DBS)?** STATE the bullets on the slide. EXPLAIN why DBS samples are useful for re-testing. They are: ▪ Easy to collect ▪ Easy to store ▪ Easy to transport
3	**EQA Re-testing** REMIND participants that two types of specimens may be collected at the site for referral to a reference lab for re-testing: serum/plasma or dried blood spots (DBS). This module focuses on DBS. REVIEW EQA re-testing.
4	**Learning Objectives** STATE the objectives on the slide.
5	**Content Overview** EXPLAIN the topics that will be covered in this module.
Flipchart 	WRITE the content outline on a flipchart prior to training. REFER to it frequently to orient participants to where they are in the module.
6	**What Are Your Responsibilities?** STATE the tester's (or lab technician's) job responsibilities related to collecting and handling EQA specimens. EMPHASIZE the importance of the specimen quality – a test result is only as good as the specimen collected.

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7	**<u>EQA Specimen Transfer Log</u>** REFER participants to form in their manual. EMPHASIZE the importance of accurate data entry. PROVIDE examples of typical transcription errors. • Care should be taken in transferring information from test records to EQA transfer log. Errors that are typically made include: • Specimen ID on transfer log sheet doesn't match specimen ID filter paper • Final result at testing site incorrectly transcribed from test records
8	**<u>Required Supplies for DBS</u>** DESCRIBE the supply items on the slide and what each is used for. EMPHASIZE that while the collection card may include 5 circles, only one patient/client's blood may be collected on one card. MENTION other supplies required: ▪ Safety supplies including discard bins ▪ Supplies for performing a fingerprick
Activity **3 minutes**	PASS around the supply items for all participants to examine.
9	**<u>How to Collect DBS</u>** REVIEW Universal Safety Precautions. ASK "What are some key safety practices you need to keep in mind when collecting blood?" WRITE their answers on a flipchart. ENSURE the following items are covered: ▪ Treat all blood samples as though they are infectious ▪ Wash hands ▪ Wear gloves and apron/lab coat ▪ Take precaution to avoid needle injury ▪ Dispose of contaminated sharps and waste appropriately

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	EMPHASIZE the importance of labeling all blood collection cards. It is unacceptable to submit a blood card for testing that has not been properly labeled. EMPHASIZE AGAIN that while the collection card may include 5 circles, only one patient/client's blood may be collected on one card.
	STATE DBS collection is usually done through finger prick. REVIEW finger prick procedures ASK participants to recall the steps in the procedures. REFERENCE the job aid for finger prick.
	DESCRIBE the procedure for collecting DBS. • Apply gentle pressure to the finger and allow a large drop of free flowing blood to collect at the puncture site. • Working quickly, hold the filter paper by the edges and touch the filter paper gently against the large drop of blood and in one step allow a sufficient quantity of blood to soak through and completely fill or saturate a circle. A completed saturated spot will contain 100 µl of blood. • Repeat, until you have collected enough blood to fill at least 3 circles on the blood collection card. • Completing filling the circle is important because the laboratory will need to use a hole puncher to punch a section of the circle of blood for testing • If collecting spots using a pipette, collect 100 µl of blood and gently apply to filter paper. ADD the following tips: • DO NOT press the filter paper against the puncture site. • Apply blood to only one side of the filter paper. • Do not layer successive drops of blood or apply blood more than once in the same collection circle. ▪ Do not "milk" the finger as excessive milking or squeezing the puncture site might cause hemolysis of the specimen or result in collection of tissue fluids with the specimen, which might adversely affect the test result. (NOTE: "*hemo-*" means red cells, "*-lysis*" means destruction, "*hemolysis*" means destruction of red cells.) INFORM participants they are going to practice at the end of the module.

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10	**<u>How to Dry DBS</u>** EXPLAIN the DBS should be dried at room temp horizontally. In addition to keeping away from direct sunlight, the spots should be protected from dust and in some cases flying insects. Care should be taken to avoid exposing DBS to environmental conditions that may compromise the integrity of the specimen. An example might be drying the spots near an open window. Why? Sunlight and dust may come in contact with the DBS during the drying procedure.
11	**<u>Dry Completely Before Packaging</u>** PROVIDE the following points: ▪ There are several ways in which you can effectively dry DBS ▪ The image on the left is one that was taken in India. DBS are dried horizontally between bamboo. ▪ The image on the right can be obtained from Schleicher & Schuell Bioscience, Inc. (Manufacturer of DBS collection cards) • DBS change from bright red to dark red as they dry.
12	**<u>How to Package DBS for storage</u>** PROVIDE an overview of the steps.
Demonstration **13-17, 19**	DEMONSTRATE each step with actual items as you show each slide.
13	**<u>1. Stacking DBS</u>** EXPLAIN placing weighing or glycine paper between DBS cards before transport prevents cross-contamination.
14	**<u>2. Insert Into Sealable Plastic Bag</u>** STATE you can typically place up to 15 DBS cards in each plastic bag. The bag should be just the right size to hold the cards. Avoid using bags that are too big as the cards will shuffle inside the bag. EMPHASIZE that sandwich bags will not work as many of these are of the type where you fold the top. The bag should be a sealable heavy duty plastic bag, one that will prevent moisture from entering.

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15	**<u>3. Add Desiccant Packets</u>** Desiccant packets serve as a drying agent. STATE at least 5 desiccant packs should be placed in each bag. Some sites may need more or fewer packets in each bag depending on environmental and storage conditions. POINT OUT that desiccant packets vary in size. The ones seen here are the smaller ones.
16	**<u>4. Add Humidity Cards and Seal Bag</u>** STATE - Often humidity cards must be recharged before use. EXPLAIN what to do if the humidity card is pink at the 30% level. ▪ Recharge card and desiccant pack by heating at 50-60°C for 3-4 hours in a drying oven. ▪ Cool 10 minutes. ▪ IMMEDIATELY RETURN card and desiccant pack to sealable plastic bag. ▪ Note: If a drying oven is not available, place cards and excessive number of desiccant packets in a sealable bag or envelope
17	**<u>5. Label Outside of Plastic Bag with Contents</u>** STATE permanent marker should be used to clearly label the contents on the bag.
18	**<u>How to Store DBS</u>** STATE the points on the slide. It is also acceptable to store DBS in a Styrofoam box. Avoid placing spots in an malfunctioning refrigerator where water may drip on or soak the spots
19	**<u>How to Package DBS for Shipping</u>** DESCRIBE the steps on the slide. POINT OUT the sealable plastic bag has a label on the outside indicating bio-hazardous contents.
20	**<u>EQA Specimen Transfer Log</u>** STATE the transfer log is sent out with the specimens.

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 *TIPS* **21-30**	Use interactive teaching technique for these slides. Facilitate group discussions about what makes a specimen valid or invalid. For example, when showing an invalid DBS specimen, ask: • "Why do you think this specimen is not acceptable?" • "What do you think may have caused it to become invalid?"
21	**<u>Valid DBS Specimen</u>** EXPLAIN the specimen is valid because: • 100 µl of blood has been collected in each circle completely saturating or filling the circle • The filter paper card has been labeled with appropriate identification. • If you look at the other side of the card, you will see blood soaked through to the other side.
22	**<u>Valid DBS Specimen</u>** DISCUSS why this specimen is valid: ▪ On the card with MB/KP/120, the blood is spreading from one circle to another due to the anemia (anemic blood is more fluid) ▪ This is still considered a valid specimen. Blood has completely filled the circle. POINT OUT to the participants that the third and fifth circles have been punched (hence the white area in the middle).
23	**<u>Invalid DBS Specimen</u>** DISCUSS why this specimen is invalid – insufficient quantity for testing. DISCUSS what may have caused it: • Removing filter paper before blood has completely filled circle or before blood has soaked through to the other side • Applying blood to filter paper with a capillary tube • Filter paper coming in contact with gloved or ungloved hands or substances such as hand lotion or powder, either before or after blood specimen collection

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24	**<u>Invalid DBS Specimen</u>** DISCUSS why this specimen is invalid – specimen appears scratched or abraded. DISCUSS what may have caused it – applying blood with a capillary tube or other device.
25	**<u>Invalid DBS Specimen</u>** DISCUSS why this specimen is invalid – specimen not dry before mailing. DISCUSS what may have caused it – mailing specimen before drying for a minimum of 4 hours.
26	**<u>Invalid DBS Specimen</u>** DISCUSS why this specimen is invalid – specimen appears clotted or layered. DISCUSS what may have caused it: • Touching the same circle on the filter paper to blood drop several times • Filling circle on both sides of filter paper The volume of specimen will not be uniform between spots resulting in errors during the testing process.
27	**<u>Invalid DBS Specimen</u>** DISCUSS why this specimen is invalid – specimen appears hemolyzed, discolored, or contaminated. DISCUSS what may have caused it: • Squeezing or “milking” of area surrounding the puncture site • Allowing filter paper to come in contact with glove or ungloved hands or substances either before or after blood collection • Exposing blood spots to direct heat

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28	**<u>Invalid DBS Specimen</u>** DISCUSS why this specimen is invalid. The specimen exhibits serum rings, in other words, serum becomes separate from cells. DISCUSS what may have caused it: • Not allowing alcohol to dry at puncture site before making skin puncture • Allowing filter paper to come in contact with alcohol, hand lotion, etc. • Squeezing area surrounding puncture site excessively • Drying specimen improperly • Applying blood to filter paper with a capillary tube
29	**<u>Invalid DBS Specimen</u>** DISCUSS why this specimen is invalid – no blood. DISCUSS what may have caused it – failure to obtain blood specimen.
30 **Demonstration** **10 Minutes**	**<u>DBS Collection: Demonstration</u>** PROVIDE an overview of the steps. REFERENCE the fingerprick steps using the job aid. REMIND participants of the tips DEMONSTRATE the DBS procedure step by step.
 *TIPS*	Depending on the number of participants you have and the number of instructors available, you may want to break the participants into smaller groups for the demonstration. When demonstrating, • Show each step slowly and methodically. • Talk out loud as you perform, but keep your statements brief. • Repeat the procedure a few times, making sure each time you do exactly the same thing so you don't confuse participants. • Share tips from your experience.

Slide Number	Teaching Points
31 **Practice** **35 minutes**	**<u>DBS Collection: Hands-on Practice</u>** CONDUCT the practice session. INFORM participants what they are going to do. DISTRIBUTE to all participants the supplies required to perform DBS collection. POINT OUT the instructions on the slide. SIGNAL when it is time to switch (every 15 minutes). MONITOR the practice and provide necessary assistance to participants. DEBRIEF by: • POINTING out the commonly-made mistakes you have observed during the practice session. • ASKING participants to share their experience and key learning from the practice. • REASSURING participants that they will improve as they gain more experience
 *TIPS*	Be prepared to provide plenty of personal attention to participants during hands-on practice. This is particularly important when teaching people without health or lab background. Set up the groups in such a way that you are able to monitor 3 or 4 groups at a time.
32 **Exercise** **5 minutes**	**<u>Exercise: Valid vs. Invalid DBS</u>** LAY on a table all the DBS samples collected from the hands-on practice. ASK participants to examine these samples, determine whether each is valid or not, and write their answers on a piece of paper. DISCUSS the answers as a group.
33	**<u>Summary</u>** ASK participants to answer the questions on the slide. ANSWER any questions participants may have.